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Use of multiplex polymerase chain reaction test in hospitalized newborns with acute respiratory tract infection: a randomized controlled trial

Aslı Okbay Güneş[1,2], Şenay Coşkun[1,2], Suat Burak Köksal[1], Kubilay Kutlucan[1], İrem Ünal[2], Şirin Güven[3]

[1]Division of Neonatology, Department of Pediatrics, Prof. Dr. Feriha Öz Emergency Hospital, İstanbul, Turkiye
[2]Division of Neonatology, Department of Pediatrics, Sancaktepe Prof. Dr. İlhan Varank Training and Research Hospital, University of Health Sciences, İstanbul, Turkiye
[3]Department of Pediatrics, Sancaktepe Prof. Dr. İlhan Varank Training and Research Hospital, University of Health Sciences, İstanbul, Turkiye

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Corresponding Author: Aslı Okbay Güneş, asliokbay@gmail.com

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ABSTRACT

Aims: We aimed to investigate whether the use of multiplex polymerase chain reaction (PCR) test reduced the duration of hospital stay, duration of antibiotic use and total hospital costs in neonates with acute respiratory tract infection (RTI).

Methods: This was a retrospective cross-sectional study including newborns with a diagnosis of RTI. We included two groups to compare the durations of antibiotic treatment, lengths of hospital stay, and total hospital costs. The first group, referred to as the "respiratory tract panel (RTP) group," consisted of our patients who underwent RTP testing. For comparison, a "non-RTP group" was created by simulating the same patients as if they had not undergone RTP testing based on the recommendations of the national neonatal society for managing similar cases.

Results: Among 76 neonates, 20 (26.3%) of the patients were diagnosed with viral RTI and 23 (30.3%) were diagnosed with bacterial RTI according to RTP. No causative agent in the etiology of RTI was detected in 33 (43.4%) newborns. No significant difference was found when the RTP group and the non-RTP group were compared in terms of duration of antibiotic use and length of hospital stay. Total hospital costs were higher in the RTP group compared to the non-RTP group (22.700 [15.890-26.105] TRY vs. 20.430 [13.620-23.835] TRY, p=0.009).

Conclusion: The use of multiplex PCR test did not have effect on the duration of antibiotic use and hospital stay in neonatal RTI. However, total hospital costs increased. Therefore, RTP test should be used selectively, especially in developing countries.

Keywords: Bacterial agents, hospital costs, neonates, respiratory tract infections, viruses

INTRODUCTION

Acute respiratory tract infection (RTI) in the infancy period is a significant cause of morbidity and mortality, and surviving patients may face problems such as recurrent wheezing and asthma in the long-term.[1-3] Newborns, especially premature babies, are more susceptible to infections, including RTI, because their immune systems are not yet fully mature.[3,4] Symptoms of RTI can vary widely, from mild symptoms such as rhinnorrhea, nasal congestion and cough to severe respiratory failure requiring mechanical ventilation and even extracorporeal membrane oxygenation.[3] On the other hand, it is difficult to predict how the disease will make progress in the neonatal period, and even nasal congestion, which seems to be a simple finding due to upper RTI, is an important problem for neonates as it may cause difficulty in sucking, feeding and sleeping.[3]

Neonatal RTI can mimic bacterial sepsis by causing temperature instability, neurological signs, apnea, gastroenteritis, skin lesions, etc. according to the underlying microbiological agent.[1,3,5-7] A respiratory tract virus was detected in 8% of high-risk infants who were evaluated for late-onset sepsis in a neonatal intensive care unit (NICU) and initiated on antibiotic treatment, and in 6% of sepsis evaluations overall.[7] In another study, the detection rate of respiratory tract viral infection in newborns with suspected nosocomial bacterial sepsis was found to be 10%.[5] It has also

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been shown that premature newborns with virus detected in periodic respiratory tract panels (RTP) have longer hospital stays and mechanical ventilation requirements compared to those with negative RTPs, and bronchopulmonary dysplasia is more common in these newborns.[8]

Multiplex polymerase chain reaction (PCR) is a sensitive and specific test for revealing RTI agents from respiratory secretions, but the use of this test has not been shown to shorten the duration of hospitalization or to reduce the frequency of antibiotic usage or general hospital cost in the childhood period.[9] Although expensive, multiplex-PCR methods can test presence of many pathogens simultaneously in a single specimen in a short period of time with a confirmation rate of over 80% in children with RTI symptoms. However, the tests may not reflect the ability of the pathogens to multiply or infectivity as the results might be positive several days after the virus can no longer be isolated by culture.[9,10] As there is no consensus on when to use multiplex-PCR, it may be feasible to use this test only if the results can change the clinical course such as when deciding using antibiotics or when deciding hospitalization.[9] In newborns admitted to the NICU due to RTI, the effect of the multiplex PCR testing on the duration of hospital stay, duration of antibiotic use and total hospital costs is unknown. In this study, we aimed to investigate whether the use of multiplex PCR test reduced the duration of hospital stay, duration of antibiotic use and total hospital costs in neonates with RTI in a level II NICU in a developing country.

METHODS

This was a retrospective cross-sectional study. Newborns who were admitted to the NICU with a diagnosis of RTI between September 2023 and June 2024 were included in the study. Ethical approval for the study was obtained from the Şehit Prof. Dr. İlhan Varank Training and Research Hospital Clinical Researches Ethics Committee (Date: 19.08.2024, Decision No: 2024/237). The study was completed in accordance with the Declaration of Helsinki as revised in 2013. Since the unit where the study was conducted was a level II NICU, newborns requiring invasive mechanical ventilation due to respiratory failure were referred to a level III NICU and were excluded from the study. Another exclusion criteria was the presence of major congenital anomalies and/or congenital heart disease. Since there was no delivery room in the center where the study was conducted, all patients were sent to our unit by a ground ambulance from other hospitals with the diagnosis of RTI. Among the patients hospitalized due to RTI, those with respiratory failure, auscultation findings (wheezing, rales, rhonchus) and appearance compatible with bronchiolitis (flattening of the ribs, increased aeration, perihilar fullness) or pneumonia (consolidation, infiltration) on the chest radiograph were diagnosed with lower RTI.[3] Upper RTI was defined as the presence of respiratory symptoms such as rhinnorrhea, nasal congestion, cough and sneezing without signs of lower RTI.

Respiratory Tract Infection Management and Follow-up Policy

Due to the fear of rapid deterioration, we monitored the patients with any signs of RTI in the neonatal period in the NICU for observation for at least 48 hours, even if only upper RTI signs were present. RTP was studied in all newborns who were admitted to our unit due to RTI findings within the first 24 hours of admission, and the results were obtained within 6 hours. The specimens were obtained by sterile flexible swabs which were inserted in each nostril and posterior nasopharynx, and a RTP was sent using a viral nucleic acid buffer transfer tube (vNAT®). This panel tests for 24 respiratory pathogens using the real-time multiplex PCR method, including *Mycoplasma pneumoniae, Bordatella pertussis*, Respiratory syncytial virus A/B (RSV), Parainfluenza virus types 1-4, Influenza A and B, Human metapneumovirus, Coronavirus (types OC43, 229E, NL63, and HKU1), Adenovirus, Human bocavirus, Human parechovirus, *Haemophilus influenzae, Legionella pneumophila, Streptococcus pneumoniae*, Rhinovirus/Enterovirus, and SARS-CoV-2.

In the presence of RTI, antibiotic treatment of the patients with viral agents detected in the RTP was discontinued, and antibiotic treatment of the patients with bacterial agents was decided according to the agent detected. If antibiotic treatment had not been started, the treatment decision was made according to the RTP results. The treatment of the patients whose RTP showed no causative agent despite RTI findings was planned by the consultant neonatologist according to the national neonatal society quideline.

All patients diagnosed with RTI underwent nasal irrigation with physiological saline whenever necessary. The patients, who were diagnosed with lower RTI, were administered 2 ml inhaled hypertonic saline 4-8 times a day, depending on the patient's condition. Inhaled salbutamol treatment was given to selected subjects whose auscultation findings worsened under inhaled hypertonic treatment.[11]

Respiratory failure was defined as presence of ongoing signs of respiratory distress and associated acidosis (pH<7.25), hypercarbia (partial pressure of arterial carbon dioxide [pCO_2] >60 to 65 mmHg), and hypoxemia (fraction of inspired oxygen [FIO_2]>40%). Intubation was reserved for neonates with respiratory failure and severe apnea on maximal nasal intermittent positive pressure ventilation (positive end-expiratory pressure ≥8 cm H_2O and positive inspiratory pressure ≥25 cm H_2O) support.[12] The newborns, who required intubation, were transferred to a level III NICU by ground ambulance.

The national guideline on neonatal infections-diagnosis and treatment for sepsis evaluation was used to create a second group for comparing the outcomes of our patients.[13] The first group, referred to as the "RTP group," consisted of our patients who underwent RTP testing upon admission. For this group, the data on the duration of antibiotic treatment, length of hospital stay, and total hospital costs were collected retrospectively. For comparison, a "non-RTP group" was created by simulating the same patients as if they had not undergone RTP testing. In the non-RTP group, the duration of antibiotic treatment, length of hospital stay, and total hospital costs were estimated based on the recommendations of the national neonatal society for managing similar cases. The outcomes for the RTP and non-RTP groups were then compared. According to the national neonatal society's guidelines, neonates with lower RTI were assumed to recieve

seven days of antibiotic therapy with a minimum hospital stay of seven days, and the total cost was calculated accordingly.

Statistical Analysis

The Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Data were assessed for normality using histogram plots and the Shapiro-Wilk test. Variables with a normal distribution were expressed as means and standard deviations (SD), while non-normally distributed variables were reported as medians and interquartile ranges (IQR) (25th and 75th percentiles). Parametric variables were compared using the Student's t-test, whereas nonparametric variables were analyzed with the Mann-Whitney U test. Categorical variables were assessed using the Chi-square test or Fisher's exact test, as appropriate. Both statistically significant and nonsignificant values were presented with their corresponding 95% confidence intervals. A p-value of less than 0.05 was considered indicative of statistical significance.

RESULTS

During the study period, 79 neonates were eligible for enrollment, three neonates were excluded from the study as two of them needed intubation and one was diagnosed with atrioventricular septal defect in the follow-up, and 76 neonates were analyzed. The median gestational age of the neonates and the postnatal age on admission were 39 (38-40) week and 18 (13-24.2) day, respectively. The mean weight of the neonates on admission were 3187±519 grams. Two (2.5%) patients needed non-invasive ventilation support and five (6.3%) needed oxygen support. Thirty-two (42.1%) of the patients had upper RTI and 44 (57.9%) had lower RTI. No growth was detected in any blood culture, and all patients were discharged with recovery. Demographic and clinical characteristics, and laboratory results are summarized in Table 1.

Table 1. Demographic and clinical characteristics, and laboratory results (n=76)

Demographic characteristics	
Female[a]	29 (38.2)
Cesarean section[a]	42 (55.3)
Gestational age, w[b]	39 (38-40)
Birth weight, g[c]	3187±519
Postnatal age on admission, day[b]	18 (13-24.2)
Clinical characteristics	
Upper RTI/lower RTI[a]	32 (42.1)/44 (57.9)
Duration of antibiotic treatment[b]	5 (3-7)
Length of hospital stay[b]	7 (5-10)
Laboratory results during sepsis	
White blood cell,/μL[b]	10.280 (8.512-13.070)
Neutrophil,/μL[b]	3.090 (2.355-5.243)
Lymphocyte,/μL[b]	5.130 (4.065-6.395)
C-reactive protein, mg/l[b]	1.64 (0.60-8.42)
Procalcitonin, μg/l[b]	0.13 (0.10-0.22)

a: Number (percentage), b: Median (interquartile range), c: Mean±standard deviation, RTI: Respiratory tract infection

According to RTP, 20 (26.3%) of the patients were diagnosed with viral RTI and 23 (30.3%) were diagnosed with bacterial RTI. No causative agent in the etiology of RTI was detected in the RTP in 33 (43.4%) patients. The most common viral agent detected in RTP was RSV (n, %=9, 21) and the most common bacterial agent was *Streptococcus pneumoniae* (n, %=3, 30.2), both causing primarily lower RTI. The causative agents of RTI detected on RTP are summarized in Table 2.

Table 2. Respiratory tract infection agents detected on respiratory tract panel (n=43)

Viral agents (n=20)	**Upper RTI (n, %=7, 16.3)**	**Lower RTI (n, %=13, 30.2)**
Respiratory syncytial virus	2 (4.7)	7 (16.3)
Influenza virus type B	1 (2.3)	1 (2.3)
Human bocavirus	2 (4.7)	0 (0.0)
Coronavirus	0 (0.0)	4 (9.3)
Enterovirus	1 (2.3)	1 (2.3)
Adenovirus	1 (2.3)	0 (0.0)
Bacterial agents (n=23)	**Upper RTI (n, %=7, 16.3)**	**Lower RTI (n, %=16, 37.3)**
Streptococcus pneumoniae	5 (11.6)	8 (18.6)
Hemophilus influenza	2 (4.7)	6 (14.0)
Bordetella pertussis	0 (0.0)	2 (4.7)

RTI: Respiratory tract infection

The platelet count was found to be lower in viral RTI compared to in bacterial RTI (p=0.040), but platelet counts were within the normal range in both bacterial and viral RTI. Duration of antibiotic treatment and length of hospital stay were longer in bacterial RTI compared to viral RTI (p=<0.001, 0.027, respectively). Comparison of laboratory and clinical findings according to the underlying microorganisms are summarized in Table 3.

Table 3. Comparison of laboratory and clinical findings according to the underlying microorganisms (n=43)

Variables	**Viral**	**Bacterial**	**p value**
White blood cell,/μL[c]	11.579±3.50	12.053±4.10	0.688[*]
Neutrophil,/μL[c]	4.096±2.24	4.601±3.09	0.819[**]
Lymphocyte,/μL[c]	5.566±2.02	5.701±2.96	0.511[**]
Hemoglobin, g/dl[c]	14.45±2.43	13.66±2.03	0.257[*]
Platelet,/μL[c]	359.895±140.93	459.609±159.50	**0.040**[*]
C-reactive protein, mg/l[b]	1.59 (0.84-2.93)	1.54 (0.60-8.22)	0.883[**]
Procalcitonin, μg/l[b]	0.13 (0.10-0.19)	0.13 (0.08-0.19)	0.494[**]
Upper RTI/lower RTI[a]	7 (16.3)/13 (30.2)	7 (16.3)/16 (37.2)	0.750[***]
Fever, yes[a]	9 (20.9)	12 (27.9)	0.639[***]
Duration of antibiotic treatment[b]	4.50 (0-5)	7 (6-10)	**<0.001**[**]
Length of hospital stay[b]	7.00 (4.75-8.50)	10.00 (7.00-10.50)	**0.027**[**]

a: Number (percentage), b: Median (interquartile range), c: Mean±standard deviation, RTI: Respiratory tract infection, p values marked with corresponding symbols are calculated with (*) the Student t-test, (**) the Mann-Whitney U test, (***) the Chi-square test, and (****) the Fisher's exact test. Statistically significant p values are marked as bold.

No significant difference was found when the RTP group and the non-RTP group were compared in terms of antibiotic

treatment duration and length of hospital stay. On the other hand, total hospital costs were higher in the RTP group compared to the non-RTP group (22.700 [15.890-26.105] TRY vs 20.430 [13.620-23.835] TRY, p= 0.009) (**Table 4**).

Table 4. Comparison of durations of antibiotic treatment, lengths of hospital stay, and total hospital costs between the RTP and non-RTP groups (n=76)

Variables	RTP group	Non-RTP group	p value*
Duration of antibiotic treatment, day	5 (3-7)	7 (0-7)	0.217
Length of hospital stay, day	7 (5-10)	7 (7-7)	0.294
Total hospital cost, TRY	22.700 (15.890-26.105)	20.430 (13.620- 23.835)	**0.009**

RTP: Respiratory tract panel, TRY: Turkish lira, * Mann-Whitney U test. Statistically significant p values are marked as bold.

DISCUSSION

In our study, which we conducted to determine whether the use of multiplex PCR test affected the duration of antibiotic use, length of hospital stay and total hospital costs in neonatal RTI, we found that there was no significant difference in duration of antibiotic treatment and hospital stay between the RTP group and the non-RTP group, however total hospital costs were higher in the RTP group compared to the non-RTP group. Only a limited number of studies have examined the costs and antibiotic usage associated with multiplex-PCR for respiratory pathogens, and so far, no significant benefit has been demonstrated for this diagnostic technique in children without risk factors, and no validated algorithms currently exist for implementing this approach in clinical practice, even in children.[9]

Since we could not detect a viral or a bacterial agent in RTP in 43.4% of our patients, the fact that treatment decisions were made according to the recommendations of the national neonatology society may be the reason why we found the hospital cost to be higher in the RTP group compared to the non-RTP group with no difference in duration of antibiotic treatment and length of hospital stay. The other fact that the multiplex PCR we use in our unit is a very expensive kit to detect too many RTI agents may be another reason for the higher cost. However, it can be mentioned that the RTP test, which is an expensive test for developing countries, may increase total hospital costs without benefiting important clinical outcomes such as antibiotic use and hospital stay, and that this test should be used very selectively in centers with limited resources. The main value of multiplex PCR testing in children with RTI is to differentiate between viral and bacterial infections, guiding decisions on further investigations and antibiotic use. Despite advances in testing, its clinical utility and specific indications remain under-discussed.[14,15] Respiratory panel testing is crucial when results can guide clinical management, particularly in febrile infants under three months, immunocompromised patients, those at risk of influenza complications, and intensive care unit-admitted children.[14,15]

As expected, the durations of antibiotic treatment and hospitalization in bacterial RTI were longer compared to viral RTI, but the newborns diagnosed with viral RTI also recieved antibiotics for a median of 4.5 days. This may be because we are afraid to monitor newborns, who are a fragile population, without antibiotics, at least for a while, in the presence of accompanying clinical sepsis findings such as fever and respiratory distress and/or laboratory sepsis findings such as elevated C-reactive protein and procalcitonin. In a study of 334 NICU-admitted neonates, PCR detection of respiratory pathogens in nasopharyngeal aspirates was 10.2%, with Parainfluenza-1, human rhinovirus, parainfluenza-3, and RSV identified in decreasing frequency.[16] The main risks for detection of a respiratory patogen were increased age and rhinorrhea among those neonatal medium care unit newborns.[17] Similar to our findings, however, the clinicians were blinded to the RTP results, the frequency of antibiotic use and length of hospital stay were similar between those with pathogens detected in the respiratory tract and those without, and so the effect of the knowledge of respiratory viruses being present in respiratory samples of newborns on clinical outcomes is still in question.[16]

In the neonatal period, routine laboratory tests, such as white blood cell, C-reactive protein etc. were found to be incapable to differentiate viral infections from bacterial infections, and newborns should be evaluated for respiratory pathogens in the presence of RTI signs.[5,6] When respiratory virus surveillance was performed in the NICU, a RTI agent was detected in half of the premature newborns even when they were asymptomatic or minimally symptomatic, and the presence of viral nucleic acids in nasopharyngeal secretions was associated with many adverse outcomes.[8] Although it does not seem right to limit the use of tests that can be used for RTI surveillance to microorganisms with specific treatment in developed countries and to selected patient groups in developing countries, it would be economically reasonable.

To our knowledge, this is the first study to evaluate the effect of multiplex PCR use in neonatal RTI on the duration of antibiotic treatment, length of hospital stay, and hospital costs in a NICU in a developing country. The fact that the use of multiplex PCR increases hospital costs without reducing the duration of antibiotic use and hospital stay suggests that it would be reasonable to use cheaper kits that are limited to viruses that may affect the treatment when detected, such as influenza virus, and to the most common bacterial agents, in newborns with signs and symptoms of RTI. It is important to organize the treatment and follow-up of all newborns diagnosed with RTI and monitored in the NICU, regardless of the RTI agent, by taking respiratory tract isolation measures. Similar to the literature, the most frequent viral RTI agent detected in our study was RSV, and it is known that RSV infection is the leading cause of hospitalization due to a severe course of RTI in infants younger than 6 months.[18]

Limitations

The main limitation of our study was the absence of a real control group. As, RTP test was a routine test for neonates with RTI in our unit, it was not possible to create a non-RTP group for real. The other limitations were the cross-sectional retrospective design of the study and small number of newborns included in the study. The final limitation was that the multiplex PCR kit we used in our hospital was intended

to detect a large number of RTI agents and their detection in RTP would have no effect other than increasing the cost, because most of these agents do not have specific treatment.

CONCLUSION

As a result, the use of multiplex PCR test did not affect the duration of antibiotic use and length of hospital stay in neonatal RTI. On the other hand, total hospital costs were higher in the RTP group compared to the non-RTP group. The RTP test, which is an expensive test for developing countries, seems to increase total hospital costs without benefiting important clinical outcomes such as antibiotic use and hospital stay, and this test should be used very selectively in developing countries with limited resources. Prospective studies that include more patients with a real control group may help investigate the true impact of using RTP testing on patient outcomes and hospital costs.

ETHICAL DECLARATIONS

Ethics Committee Approval

Ethical approval for the study was obtained from the Şehit Prof. Dr. İlhan Varank Training and Research Hospital Clinical Researches Ethics Committee (Date: 19.08.2024, Decision No: 2024/237).

Informed Consent

Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Aslı Okbay Güneş

Aslı Okbay Güneş was born on July 7, 1986, in Bandırma, Balıkesir, Turkiye. She completed her medical education at Marmara University Faculty of Medicine, between 2004 and 2010. Following her graduation, she completed her pediatrics residancy training at İstanbul University-Cerrahpaşa Department of Child Health and Diseases between 2011-2016, and her Neonatology subspecialty training at Zeynep Kamil Training and Research Hospital between 2017-2020. She completed her first compulsory service as a general practitioner at Kadıköy emergency health station no. 4, her second compulsory service as a pediatrician at Esenler Maternity and Children's Hospital, and her third compulsory service as a neonatologist at Şanlıurfa Training and Research Hospital. Finally, Dr. Okbay Güneş has been working as an Associate Professor at Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital since September 2023.